

Etanercept Exerts Beneficial Effects on Articular Cartilage Biomarkers of Degradation and Turnover in Patients with Ankylosing Spondylitis

WALTER P. MAKSYMOWYCH, A. ROBIN POOLE, LORI HIEBERT, ALISON WEBB, MIRELA IONESCU, TATIANA LOBANOK, LINDSAY KING, and JOHN C. DAVIS Jr

ABSTRACT. Objective. Anti-tumor necrosis factor- α (TNF- α) therapies are not only beneficial for reducing symptoms in rheumatoid arthritis (RA) but also for structural damage visible on plain radiographs and serological biomarkers of articular cartilage damage. It is not known if these therapies also prevent structural damage in ankylosing spondylitis (AS). The low sensitivity to change over time of plain radiographic instruments mandates a search for the effects of these therapies on possible biomarkers of cartilage damage.

Methods. We studied 2 populations of patients with AS: (1) patients recruited to a placebo controlled trial of etanercept in AS for 16 weeks; (2) an observational cohort receiving infliximab for disease refractory to conventional therapy. Clinical (morning stiffness, nocturnal pain, Bath AS Disease Activity Index) and laboratory [erythrocyte sedimentation rate (ESR), C-reactive protein (CRP)] assessments of disease activity were performed at baseline and at either 16 weeks (clinical trial cohort) or at 14 weeks (observational cohort). We measured serum matrix metalloproteinase-1 (MMP-1), MMP-3, human cartilage glycoprotein-39 (YKL-40), and cartilage oligomeric matrix protein by ELISA at the same timepoints. We also measured serum concentrations of 2 novel biomarker epitopes, C2C and 846, by competitive ELISA. The C2C assay detects a neoepitope at the carboxy terminus of the long three-quarter amino-terminal fragment generated following cleavage of type II collagen by collagenases. Aggrecan 846 epitope is a chondroitin sulfate epitope present on intact aggrecan molecules. Both these assays would detect products originating from both hyaline cartilages and intervertebral discs.

Results. There was a significant reduction in levels of C2C ($p = 0.005$) and a significant increase in the 846 epitope ($p = 0.01$) in patients who received etanercept compared to placebo controls. Changes in C2C correlated significantly with changes in ESR ($r = 0.51$, $p = 0.04$) and CRP ($r = 0.48$, $p = 0.048$). Significant changes in C2C were not evident in the infliximab observational cohort, although significant reductions were noted in levels of MMP-3 ($p = 0.04$) and MMP-1 ($p = 0.02$) at 14 weeks that were not observed in the etanercept group. Analysis of all baseline samples showed a significant correlation between levels of MMP-3 with CRP ($r = 0.73$, $p < 0.0001$), and YKL-40 ($r = 0.71$, $p < 0.0001$). No correlation was evident at baseline between levels of C2C or 846 epitope and either acute phase reactants or other biomarkers.

Conclusion. Our data suggest that an anti-TNF- α agent, etanercept, may modify cartilage turnover. These include decreased degradation of type II collagen and increased turnover of aggrecan. Additional therapeutic properties of some anti-TNF- α agents in AS, such as infliximab, may be related to decreased expression of MMP. Additional studies in larger populations are therefore warranted. (J Rheumatol 2005;32:1911–7)

Key Indexing Terms:

ANKYLOSING SPONDYLITIS INFlixIMAB ETANERCEPT BIOMARKERS

From the Department of Medicine, University of Alberta, Edmonton, Alberta; Joint Diseases Laboratory, Shriners Hospital for Children, McGill University, Montreal, Quebec; Division of Rheumatology, Department of Medicine, University of California, San Francisco, California, USA; and Diagnostics R&D, Ibex Technologies, Montreal, Quebec, Canada.

Dr. Maksymowych is a Senior Scholar of the Alberta Heritage Foundation for Medical Research. Prof. Poole was supported by Shriners Hospitals for Children, the Canadian Institute of Health Research, and the Canadian Arthritis Network. The placebo controlled trial of etanercept in AS was supported by the US National Institutes of Health (N-AR-0-2244). Drug, placebo, and partial study funding for the placebo controlled trial of etanercept in AS was provided by Immunex, Seattle, Washington, USA.

W.P. Maksymowych, FRCPC, FACP, FRCP(UK), Professor; L. Hiebert, BSc, Technician, Department of Medicine, University of Alberta; A.R. Poole, PhD, DSc, Professor; M. Ionescu, BSc, Technician; T. Lobanok, BSc, Technician, Joint Diseases Laboratory, Shriners Hospital; A. Webb, RN; J.C. Davis Jr, MD, Associate Professor, Department of Medicine, University of California, San Francisco; L. King, PhD, Manager, Diagnostics R&D, Ibex Technologies.

Address reprint requests to Dr. W.P. Maksymowych, 562 Heritage Medical Research Building, University of Alberta, Edmonton, Alberta T6G 2S2, Canada. E-mail: walter.maksymowych@ualberta.ca

Accepted for publication May 31, 2005.

Ankylosing spondylitis (AS) is a relatively common form of arthritis that may lead to severe functional impairment secondary to inflammation and the spinal ankylosis that is typical of this disease. Until recently, therapy was restricted to the use of physical measures that preserved spinal mobility and the use of nonsteroidal antiinflammatory agents (NSAID) that alleviate symptoms. More recently, anti-tumor necrosis factor- α (anti-TNF- α) therapies have been shown to be effective in patients whose symptoms are insufficiently controlled by NSAID^{1,2}. Although highly effective in suppressing symptoms³, acute phase reactants⁴, and histopathological features⁵ related to inflammation, there is no evidence that these therapies are disease modifying in preventing the development of plain radiographic features of AS.

A major limitation in the development of disease modifying therapies for AS has been the lack of standardized and validated radiographic instruments capable of assessing structural damage that meet the requirements of the OMER-ACT filter for feasibility, truth, and discrimination⁶. Three instruments have recently been developed that appear to be reproducible, although one, the Bath AS Radiology Index (BASRI)⁷, grades rather than scores structural damage. At least 2 years are required before change can be confirmed, and this is seen in only a small minority of patients. The Stoke AS Spinal Score (SASSS) does not score damage in the cervical spine and also shows limited sensitivity to change^{8,9}. The modified SASSS (mSASSS), which does assess damage in the cervical spine, appears to be the most responsive index currently available, although even with this instrument, 2 years are required before change is evident and only 40% of patients show evidence of progression^{10,11}. Consequently, assessment of disease modification using the mSASSS as the primary outcome would still require that placebo controlled trials be of 2 years' duration. Clearly, this is not a realistic solution for either clinical practice or research. While acute phase reactants have been shown to predict structural damage in patients with rheumatoid arthritis (RA), they are elevated in only a minority of patients with AS¹² and show poor correlation with other measures of disease activity¹³.

Similar impediments to assessment of structural damage exist in the field of osteoarthritis (OA), where recent efforts have targeted serological biomarkers of articular cartilage turnover and degradation. Several biomarkers have also been shown to have predictive validity for structural damage in both RA and OA. These include matrix metalloproteinases (MMP), especially MMP-1 and MMP-3^{14,15}, cartilage oligomeric matrix protein (COMP)^{16,17}, and human cartilage glycoprotein-39 (YKL-40)¹⁸. MMP-1 can degrade type II collagen in articular cartilage and MMP-3 can activate pro-MMP-1, while both COMP and YKL-40 are cartilage matrix proteins that are increased in states of increased matrix turnover. In general, these markers are increased in

RA and decrease following treatment with anti-TNF- α therapies^{19,20}, while the data for OA are much less consistent. Despite the involvement of cartilaginous structures in AS, biomarker studies in AS are much more limited. One report has described elevated levels of MMP-3 in AS patients with concomitant peripheral joint synovitis²¹.

Biomarkers have been developed targeting a *de novo* epitope on type II collagen generated following cleavage of the triple helix by collagenases^{22,23} and epitopes on the C-telopeptide²⁴⁻²⁶. Another targets the 846 epitope on chondroitin sulfate side-chains of aggrecan²⁷⁻²⁹. Some of these have been shown to be sensitive and specific for early cartilage changes associated with isolated joint injury in an experimental model of OA³⁰. Collagen cleavage products are increased in acute and chronic RA^{23,25,31}, whereas the 846 epitope is increased in chronic RA^{28,29}. To date there are no published reports on the use of these biomarkers to study AS.

The effects of anti-TNF- α therapies on a limited set of serological biomarkers in AS have thus far been reported in one study⁴. In that observational cohort study, there was no overall change in levels of MMP-3 and YKL-40 following infliximab therapy, although reductions were evident commensurate with reductions in clinical and laboratory markers of disease activity. We describe the effects of another anti-TNF- α agent, etanercept, on a broader spectrum of serological biomarkers in the context of a placebo controlled trial. We also describe the effects of infliximab on some of the more direct biomarkers of cartilage turnover, C2C and the 846 epitope.

MATERIALS AND METHODS

Patients. We studied 2 categories of patients. The first included patients recruited to a placebo controlled trial of etanercept in AS¹. Briefly, patients were recruited from rheumatology practices in Northern California who met the modified New York criteria for AS. Patients had active AS as defined by the presence of ≥ 45 min of morning stiffness and at least moderate disease activity as assessed by patient (5 point scale) and physician [≥ 40 mm on a 0–100 mm visual analog scale (VAS)]. Patients were required to be undergoing stable nonsteroidal antiinflammatory drug (NSAID) therapy and/or stable second-line therapy (methotrexate, sulfasalazine, intramuscular gold) and/or prednisone (≤ 10 mg/day) for ≥ 1 month prior to randomization. Patients were excluded if they had spondylitis other than AS, clinical or radiographic evidence of complete spinal ankylosis, a history of recurrent infections or cancer, or a serious liver, renal, hematologic or neurologic disorder. The study was approved by the committee on human research at the University of California, San Francisco, and by the US Food and Drug Administration. All patients provided written informed consent.

The second category of patients included an observational cohort of consecutive patients with AS receiving infliximab, as described⁴. Briefly, these patients received infliximab for disease that was refractory to NSAID and/or second-line agents and intraarticular steroids. Patients were required to maintain stable NSAID and/or second-line therapy during the treatment period. The study was approved by the University of Alberta ethics board and all patients provided written informed consent.

Study protocol. Patients in the clinical trial population were randomly assigned to receive twice-weekly subcutaneous injections of placebo or

etanercept (25 mg) for 4 months. Clinical and laboratory assessments were performed at the time of screening and on study days 1, 28, 56, 84, and 112. Serological biomarkers were assessed on Day 1 and Day 112. Patients in the observational cohort received an induction dose of infliximab 3 mg/kg at baseline, 2 weeks, and 6 weeks. Clinical and laboratory assessments were performed at baseline and at 14 weeks. Serum samples were stored at -20°C until assayed.

Outcome measures. Clinical. In the clinical trial population, disease activity was assessed by recording duration of morning stiffness (min), the degree of spinal pain at night (as represented on a 100 mm VAS, 0 mm indicating absence of pain and 100 mm the most severe pain), and the patient and physician global assessments of disease activity. In the observational cohort, disease activity was assessed using the Bath AS Disease Activity Index (BASDAI)³².

Laboratory measures. Erythrocyte sedimentation rate (ESR) was measured by the Westergren method; C-reactive protein (CRP) was measured by nephelometry (mg/dl). Serum MMP-1 and -3 were measured by ELISA (BioTrak; Amersham BioSciences, Little Chalfont, Buckinghamshire, UK). The MMP-1 kit recognizes total human MMP-1, pro-MMP-1, free MMP-1, and MMP-1 complexed with tissue inhibitor of metalloproteinase (TIMP-1), but not with α_2 -macroglobulin. The range of the assay is 6.25–100 ng/ml with sensitivity of 1.7 ng/ml. The MMP-3 assay recognizes pro-MMP-3, active MMP-3, and MMP-3/TIMP complexes, but not MMP-3 bound by α_2 -macroglobulin. The range of the assay is 3.75–120 ng/ml with sensitivity of 2.35 ng/ml. The MMP-1 and MMP-3 antibodies do not cross-react. Serum samples were diluted (1:1 for MMP-1, between 1:1 and 1:8 for MMP-3) and individual samples were assayed in duplicate. Serum COMP (Anamar Medical AB, Uppsala, Sweden) and YKL-40 (Metra™ YKL-40; Quidel Corp., San Diego, CA, USA) were measured in duplicate by ELISA. The C2C assay detects a neopeptide at the carboxy terminus of the long amino-terminal fragment generated following cleavage by collagenases²³. It is measured using a competitive inhibition monoclonal antibody-based ELISA (Ibex Technologies, Montreal, Quebec, Canada). Aggrecan 846 epitope is present on intact aggrecan molecules and is associated with chondroitin sulfate chains²². This epitope is detected by ELISA using a mouse monoclonal antibody (Ibex Technologies).

Statistical analysis. The primary analysis of the clinical trial population was a between-treatment group comparison of serum levels of individual biomarkers using Wilcoxon's matched-pairs signed-ranks test, 2-tailed. For the observational cohort, we analyzed the effects of treatment on individual biomarkers using the Wilcoxon rank-sum test, 2-tailed. Spearman's rank correlation coefficient was used to analyze the relationship between levels of individual biomarkers with clinical and laboratory indicators of disease activity. Linear regression was used to analyze the correlation between changes in individual biomarkers and changes in clinical and laboratory measures of disease activity. Statistical analysis was performed using GraphPad InStat and statistical significance was defined as $p < 0.05$. P values corrected for the number of comparisons are also shown.

RESULTS

Serum was available from 18 patients randomized to either etanercept ($n = 9$; 77% male, mean age 36.5 yrs, mean disease duration 12.3 yrs, 100% HLA-B27-positive) or placebo ($n = 9$; 88% male, mean age 39.3 yrs, mean disease duration 11.7 yrs, 100% HLA-B27-positive) for 16 weeks. The 2 groups were comparable for baseline disease characteristics (Table 1). Mean swollen joint and enthesitis scores were higher and mean ESR value lower at baseline in the placebo group, although these differences were not significant. For biomarkers, mean serum levels of MMP-3 and aggrecan 846 epitope were higher at baseline in the placebo group,

although the differences from values in the etanercept group were not significant and were related to single outliers for each biomarker in the placebo group.

A significant improvement was noted in nearly all clinical indicators and acute phase reactants for patients who received etanercept. Between-group comparisons with placebo patients also showed significant benefit for etanercept for these measures (as reported¹). Among the biomarkers analyzed, a significant reduction of 15.4% ($p = 0.005$) and an increase of 52.3% ($p = 0.01$) was observed in levels of C2C and 846 epitope, respectively, in etanercept treated patients, compared to an increase of 13.3% and a decrease of 25.1% in placebo patients (Table 1, Figure 1). Treatment group comparisons of pre- and post-treatment changes in MMP-3, MMP-1, YKL-40, and COMP concentrations revealed no significant differences.

Serum was available on 14 AS patients who received infliximab, and preliminary data describing the analysis of MMP-3 and YKL-40 in a subset of these patients have been described⁴. Eight (57.1%) of these patients had active peripheral synovitis, 3 had Crohn's disease, one had psoriasis, and one had reactive arthritis. There were 13 men and one woman (mean age 43.1 yrs, range 26–66) with mean duration of symptoms of 17.4 years (range 7–30). A significant reduction in levels of MMP-1 ($p = 0.02$) and MMP-3 ($p = 0.04$) was evident following treatment, but not in levels of other biomarkers (Table 2).

Baseline samples from both populations were compared in patients with and without concomitant peripheral synovitis. Table 3 shows that while most biomarkers were elevated in patients with concomitant peripheral synovitis, these differences did not reach statistical significance. When baseline samples from both populations were analyzed for correlations between individual biomarkers as well as between biomarkers and clinical/laboratory measures of disease activity, significant correlations were noted between levels of MMP-3 and either CRP ($r = 0.73$, 95% CI 0.51–0.86, $p < 0.0001$) or YKL-40 ($r = 0.71$, 95% CI 0.48–0.85, $p < 0.001$) that were significant when p values were corrected for the number of comparisons (Table 4). A weaker correlation was noted between levels of YKL-40 and CRP that was not significant after correction. An inverse correlation was noted between levels of C2C and MMP-3, although the corrected p value was not significant. There were no significant correlations between clinical indicators (BASDAI, morning stiffness, nocturnal pain) and individual biomarkers (data not shown).

A significant correlation was evident between change in C2C values and change in ESR for patients in the etanercept trial ($r = 0.51$, 95% CI 0.02–0.80, $p = 0.04$) (Figure 2). The correlation with change in CRP was of borderline significance ($r = 0.48$, 95% CI -0.02 to 0.79, $p = 0.048$). No significant correlation was evident between change in C2C levels and change in either clinical indicators or other bio-

Table 1. Pre- and post-treatment scores for clinical and laboratory indicators in patients with AS randomized to placebo or etanercept for 16 weeks.

Indicator	Placebo, n = 9		Etanercept, n = 9	
	Baseline	16 Weeks	Baseline	16 Weeks
Mean AM stiffness (SD), min	110.6 (97.4)	91.1 (46.3)	107.2 (57.3)	71 (112.4)
Mean nocturnal pain (SD)	51.7 (26.7)	45.6 (22.1)	50.9 (24.1)	18.3 (15.1)
Mean patient global (SD)	3.2 (0.7)	3.0 (0.7)	3.2 (0.7)	2.6 (0.5)
Mean BASFI (SD)	4.7 (2.7)	5.0 (2.7)	5.1 (1.7)	2.3 (1.5)
Mean swollen joint score (SD)	5.0 (7.1)	6.4 (10.8)	2.0 (4.6)	0.6 (1.1)
Mean enthesitis index (SD)	8.9 (10.5)	7.8 (11.0)	4.7 (5.5)	0.8 (1.4)
Mean CRP, mg/dl (SD)	1.5 (0.7)	2.2 (3.5)	1.8 (1.2)	0.7 (0.4)
Mean ESR, mm/h (SD)	19.8 (14.3)	19.1 (14.6)	31.1 (24.9)	7.0 (6.3)
Mean MMP-1, ng/ml (SD)	16.8 (10.3)	17.1 (10.3)	17.5 (15.6)	17.8 (13.9)
Mean MMP-3, ng/ml (SD)	63.7 (132.0)	51.6 (92.5)	19.7 (7.4)	15.5 (11.5)
Mean YKL-40, ng/ml (SD)	58.9 (38.1)	73.7 (79.1)	69.3 (28.9)	57.3 (30.5)
Mean COMP, U/l (SD)	12.3 (5.0)	10.5 (2.6)	11.0 (2.3)	10.0 (1.9)
Mean C2C epitope, ng/ml (SD)	50.5 (31.2)	52.2 (34.4)	41.9 (11.9)	33.7 (7.4)*
Mean aggrecan 846 epitope, ng/ml (SD)	820.5 (857.5)	302.2 (206.5)	349.1 (178.6)	506.4 (284.7)*

* p < 0.05.

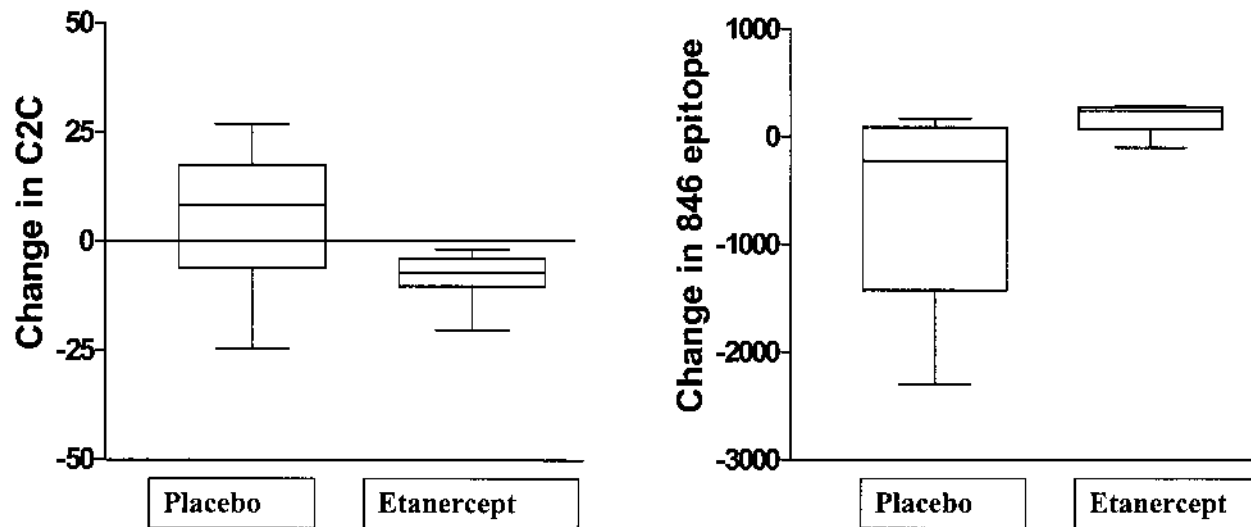


Figure 1. Change in serum levels of biomarkers C2C and 846 epitope after 16 weeks in patients randomized to either placebo (n = 9) or etanercept (n = 9). Values represent absolute change (ng/ml); plots show medians, 25th/75th centiles, minimum and maximum values.

Table 2. Pre- and post-treatment values for clinical and laboratory indicators in an observational cohort of patients with AS receiving infliximab at baseline, 2 weeks, and 6 weeks.

Indicator	Infliximab Patients, n = 14	
	Baseline	14 Weeks
Mean BASDAI (SD)	6.3 (1.5)	2.8 (2.1)
Mean ESR (SD)	31.7 (15.9)	13.4 (13.6)
Mean CRP (mg/dl) (SD)	51.4 (52.3)	24.3 (48.5)
Mean MMP-1 (SD)	10.0 (6.8)	6.6 (3.1)*
Mean MMP-3 (SD)	272.0 (272.3)	153.0 (210.2)*
Mean YKL-40 (SD)	204.8 (148.3)	188.6 (211.5)
Mean COMP (SD)	18.3 (10.4)	21.2 (10.4)
Mean C2C epitope (SD)	67.0 (19.5)	66.6 (22.0)
Mean aggrecan 846 epitope (SD)	641.9 (529.0)	690.6 (942.6)

* p < 0.05.

markers (data not shown). There was no correlation between change in levels of 846 epitope and changes in either clinical indicators, acute phase reactants, or other biomarkers (data not shown). Analysis of data from patients who received infliximab did not reveal significant correlations between changes in C2C and acute phase reactants, although this was evident for changes in levels of MMP-3 ($r = 0.68$ for correlation with Δ CRP, $p = 0.008$; $r = 0.58$ for correlations with Δ ESR, $p = 0.04$) and YKL-40 ($r = 0.58$ for Δ CRP, $p = 0.03$).

Table 3. Biomarker concentrations at baseline in patients with AS according to peripheral joint inflammation.

Biomarker	Peripheral Arthritis (+), mean (SD) (n = 16)	Peripheral Arthritis (–), mean (SD) (n = 16)
CRP	43.2 (55.3)	21.0 (23.1)
ESR	25.4 (16.8)	30.4 (20.3)
MMP-3	171.4 (205.6)	113.6 (240.7)
MMP-1	15.3 (12.6)	12.0 (8.8)
YKL-40	167.0 (155.6)	84.4 (53.5)
COMP	16.1 (7.7)	14.1 (8.2)
C2C	56.2 (29.0)	49.7 (24.0)
846 epitope	504.0 (318.8)	562.6 (625.1)

DISCUSSION

Our analysis is the first to show in a placebo controlled trial that the anti-TNF- α agent etanercept may also influence cartilage and/or intervertebral disc metabolism in a manner suggestive of a protective effect by etanercept on type II collagen degradation and an increase in aggrecan turnover. The 846 epitope is most elevated in growing tissues, where we believe it is reflective of active aggrecan synthesis^{27,33}. Specifically, using 2 assays that are highly specific for epitopes within articular cartilage and intervertebral discs, we show decreased levels of C2C, a *de novo* epitope generated following cleavage of type II collagen by collagenase, and increased levels of the aggrecan-specific 846 epitope, suggesting decreased degradation and increased synthesis of matrix components, respectively.

Although our findings were evident even in a small group of patients they will require confirmation in a larger dataset. Nevertheless, our data do appear to be internally consistent. While the C2C antibody detects collagenase cleavage of type II collagen, the 846 antibody recognizes the epitope only on the largest proteoglycan molecules in human cartilage³⁴. Because aggrecan is susceptible to degradation, the 846 epitope likely signifies the presence of more recently

Table 4. Spearman correlation analysis of cartilage biomarkers and acute phase reactants at baseline in patients with AS. P values are shown corrected for numbers of comparisons.

	MMP-3	YKL-40	COMP	C2C	846 Epitope	CRP	ESR
MMP-1	–0.26	–0.10	–0.06	–0.56**	–0.10	–0.21	–0.28
MMP-3	—	0.71***	0.20	–0.03	0.25	0.73***	0.29
YKL-40	—	—	0.02	–0.16	–0.11	0.38*	–0.13
COMP	—	—	—	0.16	–0.09	–0.21	–0.21
C2C	—	—	—	—	–0.18	0.33	0.11
846 epitope	—	—	—	—	—	0.37	0.17

* $p = 0.04$, ** $p = 0.008$, *** $p < 0.0001$.

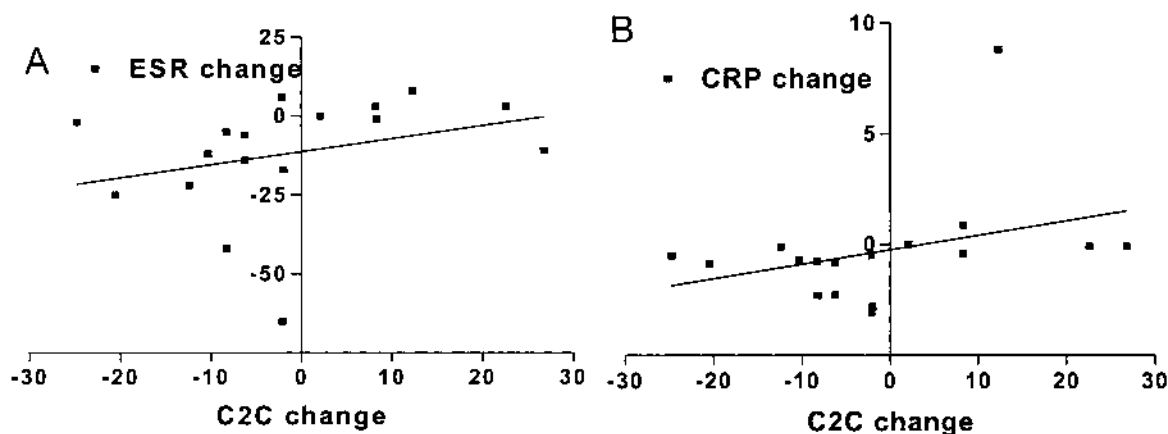


Figure 2. Linear regression analysis of correlation between change in serum levels of C2C and ESR (A) and CRP (B) in patients receiving either etanercept (n = 9) or placebo (n = 9) for 16 weeks.

synthesized molecules²². It is therefore reasonable to expect that effective treatment would be associated with divergent effects on these 2 biomarkers. In addition, a reduction in levels of C2C following treatment with etanercept correlated with a reduction in acute phase reactants, suggesting a relationship to the control of inflammation.

We did not observe these findings in the cohort of patients who received infliximab, but instead noted rather significant reductions in MMP-1 and -3, the latter correlating with changes in CRP and YKL-40. The reductions in MMP-1 and -3 were of borderline significance in our earlier report for the infliximab cohort, likely reflecting type II error with small sample size⁴. These reductions were not observed with etanercept. This may simply reflect an inadequate sample population. However, infliximab treated patients had higher levels of C2C at baseline (median value 61.7) compared to those recruited to the clinical trial (median value 39.1). This was also evident for levels of 846 epitope (median values of 426.1 and 313.2 for patients in the observational cohort and clinical trial, respectively), CRP, MMP-3, and YKL-40, suggesting more active disease and/or increased cartilage turnover/degradation in the infliximab cohort at baseline. It is therefore possible that higher doses of infliximab may have been required to exert significant effects on cartilage biomarkers than those used in these patients. However, a recent study of 23 AS patients has also described no significant effect of infliximab therapy on the same cartilage biomarkers that we used in this study³⁵.

It has also been suggested that levels of some biomarkers may vary according to the phenotype of disease in spondyloarthropathy²¹. For instance, MMP-3, but not MMP-1, has been associated with peripheral joint synovitis. Baseline levels of several biomarkers in our study tended to be elevated in patients with concomitant peripheral synovitis, although our sample size was small. MMP-3 is primarily produced by synovial cells in the setting of synovitis, while C2C directly reflects degradation of type II collagen. It is therefore possible that patients in the infliximab cohort had more active peripheral synovitis, accounting for the increased baseline levels of MMP-1 and MMP-3 and the more impressive effects of treatment on these markers. This would also be consistent with reports describing downregulation of MMP in patients with RA²⁰. The levels of several of these biomarkers have been shown to vary according to age and sex, although there were no obvious differences in age and sex between the 2 patient populations that would account for these baseline differences. Our data therefore suggest that the pattern and magnitude of effects of anti-TNF- α agents on cartilage biomarkers may depend on other factors, such as the baseline level of disease activity, phenotype of disease, and baseline level of cartilage turnover.

Although various cartilage biomarkers have been extensively studied in RA and OA, there are limited data in patients with AS. An increase in MMP-3 was noted in 2 of

3 studies where levels in AS patients were compared to control samples, although no correlation with CRP was evident^{15,36,37}. One study showed no increase in MMP-1 compared to healthy controls¹⁵. Comparative studies of COMP and YKL-40 have yet to be performed, although one study reported higher levels of YKL-40 in patients with inflammatory bowel disease and concomitant peripheral arthritis versus no peripheral arthritis³⁸. Further, levels correlated with the numbers of affected joints.

We have demonstrated that therapy with etanercept in patients with AS is associated with a significant decrease in serum levels of a neoepitope that is a highly specific marker of type II collagen degradation by collagenase, and an increase in levels of a second neoepitope that reflects increased turnover of the proteoglycan aggrecan, which may signal a reparative response. Our data support the view that these biomarkers, and other markers reflecting cartilage damage and repair, merit inclusion for further study in clinical trials of new therapeutics for AS. The clinical significance of these observations should be further examined in prospective studies of the predictive validity of these biomarkers for structural damage in patients with AS.

REFERENCES

1. Gorman JD, Sack KE, Davis JC. A randomized, double-blind, placebo-controlled trial of etanercept in the treatment of ankylosing spondylitis. *N Engl J Med* 2002;346:1349-56.
2. Braun J, Brandt J, Listing J, et al. Treatment of active ankylosing spondylitis with infliximab: a randomized controlled multicentre trial. *Lancet* 2002;359:1187-93.
3. Van den Bosch F, Kruithof E, Baeten D, et al. Randomised double-blind comparison of chimeric monoclonal antibody to tumor necrosis factor α (infliximab) versus placebo in active spondyloarthropathy. *Arthritis Rheum* 2002;46:755-65.
4. Maksymowych WP, Jhangri GS, Lambert RG, et al. Infliximab in ankylosing spondylitis: A prospective observational inception cohort analysis of efficacy and safety. *J Rheumatol* 2002;29:959-65.
5. Baeten D, Kruithof E, Van den Bosch F, et al. Immunomodulatory effects of anti-tumor necrosis factor α therapy on synovium in spondyloarthropathy: Histologic findings in eight patients from an open-label pilot study. *Arthritis Rheum* 2001;44:186-95.
6. Bellamy N. Clinimetric concepts in outcome assessment: The OMERACT Filter. *J Rheumatol* 1999;26:948-50.
7. MacKay K, Mack C, Brophy S, Calin A. The Bath Ankylosing Spondylitis Radiology Index (BASRI). A new validated approach to disease assessment. *Arthritis Rheum* 1998;41:2263-70.
8. Dawes PT. Stoke Ankylosing Spondylitis Spine Score. *J Rheumatol* 1999;26:993-6.
9. Spoorenberg A, de Vlam K, van der Linden S, et al. Radiological scoring methods in ankylosing spondylitis. Reliability and change over 1 and 2 years. *J Rheumatol* 2004;31:125-32.
10. Creemers MCW, Franssen MJAM, van 't Hof MA, Gribnau FWJ, van de Putte LBA, van Riel PLCM. A radiographic scoring system and identification of variables measuring structural damage in ankylosing spondylitis [thesis]. Nijmegen, The Netherlands: University of Nijmegen; 1994.
11. Wanders AJB, Landewe RBM, Spoorenberg A, et al. What is the most appropriate radiologic scoring method for ankylosing spondylitis? *Arthritis Rheum* 2004;50:2622-32.
12. Dougados M, Geuguen A, Nakache JP, et al. Clinical relevance of

- C-reactive protein in axial involvement of ankylosing spondylitis. *J Rheumatol* 1999;26:971-4.
13. Spoorenberg A, van der Heijde D, de Klerk E, et al. Relative value of erythrocyte sedimentation rate and C-reactive protein in assessment of disease activity in ankylosing spondylitis. *J Rheumatol* 1999;26:980-4.
 14. Green MJ, Gough AKS, Devlin J, et al. Serum MMP-3 and MMP-1 and progression of joint damage in early rheumatoid arthritis. *Rheumatology Oxford* 2003;42:83-8.
 15. Keyszer G, Lambiri I, Nagel R, et al. Circulating levels of matrix metalloproteinases MMP-3 and MMP-1, tissue inhibitor of metalloproteinases 1 (TIMP-1) and MMP-1/TIMP-1 complex in rheumatic disease. Correlation with clinical activity of rheumatoid arthritis versus other surrogate markers. *J Rheumatol* 1999;26:251-8.
 16. Vilim V, Olejarova M, Machacek S, et al. Serum levels of cartilage oligomeric matrix protein (COMP) correlate with radiographic progression of knee osteoarthritis. *Osteoarthritis Cartilage* 2002;10:707-13.
 17. Conrozier T, Saxne T, Sei Fan CS, et al. Serum concentrations of cartilage oligomeric matrix protein and bone sialoprotein in hip osteoarthritis: a one year prospective study. *Ann Rheum Dis* 1998;57:527-32.
 18. Peltomaa R, Paimela L, Harvey S, et al. Increased level of YKL-40 in sera from patients with early rheumatoid arthritis: a new marker for disease activity. *Rheumatol Int* 2001;20:192-6.
 19. Brennan FM, Browne KA, Green PA, et al. Reduction of serum matrix metalloproteinase 1 and matrix metalloproteinase 3 in rheumatoid arthritis patients following anti-tumour necrosis factor- α (cA2) therapy. *Br J Rheumatol* 1997;6:643-50.
 20. Catrina AI, Lampa J, Ernestam S, et al. Anti-tumour necrosis factor (TNF)- α therapy (etanercept) down-regulates serum matrix metalloproteinase (MMP)-3 and MMP-1 in rheumatoid arthritis. *Rheumatology Oxford* 2002;41:484-9.
 21. Vandooren B, Kruithof E, Yu DTY, et al. Involvement of matrix metalloproteinases and their inhibitors in peripheral synovitis and down-regulation by tumor necrosis factor α blockade in spondyloarthropathy. *Arthritis Rheum* 2004;50:2942-53.
 22. Poole AR. Biochemical/immunochemical biomarkers of osteoarthritis: utility for prediction of incident or progressive osteoarthritis. *Rheum Dis Clin N Am* 2003;29:805-13.
 23. Poole AR, Ionescu M, Fitzcharles MA, Billingham RC. The assessment of cartilage degradation in vivo: development of an immunoassay for the measurement in body fluids of type II collagen cleaved by collagenases. *J Immunol Methods* 2004;294:145-53.
 24. Downs JT, Lane CL, Nestor NB, et al. Analysis of collagenase-cleavage of type II collagen using a neoepitope ELISA. *J Immunol Methods* 2001;247:25-34.
 25. Garnero P, Landewe R, Boers M, et al. Association of baseline levels of markers of bone and cartilage degradation with long-term progression of joint damage in patients with early rheumatoid arthritis. *Arthritis Rheum* 2002;46:2847-56.
 26. Lohmander LS, Atley LM, Pietka TA, Eyre DR. The release of cross-linked peptides from type II collagen into human synovial fluid is increased soon after joint injury and in osteoarthritis. *Arthritis Rheum* 2003;48:3130-9.
 27. Glant TT, Kikecz K, Roughley PJ, Buzas E, Poole AR. Age related changes in protein-related epitopes of human articular cartilage proteoglycans. *Biochem J* 1986;236:71-5.
 28. Poole AR, Ionescu M, Swan A, Dieppe PA. Changes in cartilage metabolism in arthritis are reflected by altered serum and synovial fluid levels of the cartilage proteoglycan aggrecan: implications for pathogenesis. *J Clin Invest* 1994;94:25-33.
 29. Mansson B, Carey D, Alini M, et al. Cartilage and bone metabolism in rheumatoid arthritis: Differences between rapid and slow progression of diseases identified by serum markers of cartilage metabolism. *J Clin Invest* 1995;95:1071-8.
 30. Matyas JR, Atley L, Ionescu M, Eyre DR, Poole AR. Analysis of cartilage biomarkers in the early phases of canine experimental osteoarthritis. *Arthritis Rheum* 2004;50:543-52.
 31. Fraser A, Fearon U, Billingham RC, et al. Turnover of type II collagen and aggrecan in cartilage matrix at the onset of inflammatory arthritis in humans: relationship to mediators of systemic and local inflammation. *Arthritis Rheum* 2003;48:3085-95.
 32. Garrett S, Jenkinson T, Kennedy LG, Whitelock H, Gasford P, Calin A. A new approach to defining disease status in ankylosing spondylitis: The Bath Ankylosing Spondylitis Disease Activity Index. *J Rheumatol* 1994;21:2286-91.
 33. Antoniou J, Steffen T, Nelson F, et al. The human lumbar intervertebral disc: evidence for changes in the biosynthesis and denaturation of the extracellular matrix with growth, maturation, ageing, and degeneration. *J Clin Invest* 1996;98:996-1003.
 34. Rizkalla G, Reiner A, Bogoch E, Poole AR. Studies of the articular cartilage proteoglycan aggrecan in health and osteoarthritis: evidence for molecular heterogeneity and extensive molecular changes in disease. *J Clin Invest* 1992;90:2268-77.
 35. Kim TH, Stone M, Payne U, et al. Cartilage biomarkers in ankylosing spondylitis: relationship to clinical variables and treatment response. *Arthritis Rheum* 2005;52:885-91.
 36. Ribbens C, Martin y Porras M, Franchimont N, et al. Increased matrix metalloproteinase-3 serum levels in rheumatic diseases: relationship with synovitis and steroid treatment. *Ann Rheum Dis* 2002;61:161-6.
 37. Taylor DJ, Cheung NT, Dawes PT. Increased serum proMMP-3 in inflammatory arthritides: a potential indicator of synovial inflammatory monokine activity. *Ann Rheum Dis* 1994;53:768-72.
 38. Punzi L, Padswiadek M, D'Inca R, et al. Serum human cartilage glycoprotein 39 as a marker of arthritis associated with inflammatory bowel disease. *Ann Rheum Dis* 2003;62:1224-6.